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A targeted approach to developing environmental enrichment for two strains of laboratory mice

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Abstract

Previous studies on environmental enrichment have generally placed a purported enrichment in the cage and observed changes in behavioural and physiological indicators of welfare. However, many of these 'enrichments' are not purposely designed, or appear to be designed with anthropomorphic or anthropocentric concerns, which have little biological relevance. The aim of this study was to screen the behavioural responses of mice to a range of 24 potential enrichments (PEs) considered by an expert panel to have characteristics most biologically relevant to mice. Female mice (64 C57/Bl/6 and 64 ICR(CD-1)) were housed in groups of four and observed three days per week for 12 weeks whilst provided with a different PE weekly. The overall time-budgets of the two strains differed significantly. ICR(CD-1) mice performed more bar chewing, digging, manipulation of PEs, and rearing in the cage. They were also more likely to be hidden in the cage. C57/Bl/6 mice performed more drinking, cage sniffing and social interaction, and showed three times more bar climbing. The two strains responded differently to the PEs, with the ICR(CD-1) mice making more use of PEs, particularly those that could be manipulated or which provided additional hiding places. The higher trait anxiety of the C57/Bl/6 mice may have reduced their utilisation of novel PEs. We conclude that, whilst enrichment can significantly improve animal welfare, those types of enrichments that work well for all strains need to be identified, or strain-specific enrichment policies devised.

Keywords: Mice; Enrichment; Behaviour; Welfare; Housing

1. Introduction

It is now widely regarded that the limited space and lack of biologically salient opportunities in standard laboratory cages can lead to abnormal behaviours such as stereotypies (e.g., Wurbel

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et al., 1996; Wiedenmayer, 1997; Nevison et al., 1999a). Enriched cage environments permit a wider range of normal behaviour than standard cages (Haemisch et al., 1994; Nevison et al., 1999b; Sherwin, 1998; Sherwin et al., 2004) and may improve laboratory animal welfare by reducing fearfulness and anxiety (e.g., Chapillon et al., 1999; Sherwin and Olsson, 2004). However, enrichments might sometimes be designed with anthropomorphic or anthropocentric concerns such as colour, longevity and cleanliness, which could have little biological relevance for the species whose welfare they are intended to improve. In this study, we attempted to develop a suitable enrichment object by observing the behavioural responses of mice to a variety of candidate objects, using an approach similar to that adopted by van de Weerd et al. (2003). In van de Weerd's study, each of 74 objects was described using 28 descriptors and these were correlated with object-directed behaviour scores. Multiple stepwise regression analysis identified which of the characteristics played a major role in determining the level of object-directed behaviour. Van de Weerd et al., presented only one object to each of their groups, however, in the present study, several potential enrichments (PEs) were presented sequentially to the same mice.

Responses to enrichments and improvements in animal welfare might be strain-specific (e.g., Jegstrup et al., 2005; van Loo et al., 2003; Tsai et al., 2002). It is therefore important to ensure whether an enrichment is strain-appropriate and does not have inadvertent negative effects, for example, promoting fear or aggression (e.g., Nevison et al., 1999b). Enrichments that provoke strain-dependant use might be limited to improving welfare for only some strains and will therefore be of less universal applicability.

In the current study we examined the behaviour of two mouse strains, the inbred C57/Bl/6 and the outbred ICR(CD-1). The C57/Bl/6 strain is characterised as relatively neo-phobic (Cabib et al., 2002; Kalueff and Tuohimaa, 2000), so we predicted that their response to enrichment might differ from that of a widely-used outbred strain known to be less neo-phobic. Female mice of each strain were observed in their home cages under conditions of sequential exposure to a range of different types of potential cage enrichment. Their time budgets and direct responses to the potential enrichments were recorded. The aim of this study was to investigate the behavioural responses of mice to a range of potential enrichments so that we could elucidate which characteristics were most biologically relevant. These could then be incorporated into a single, potentially universal enrichment for laboratory mice.

2. Materials and methods

2.1. Animals and housing conditions

Female mice of two strains (C57/Bl/6, $n = 64$; ICR(CD-1), $n = 64$) were used. We used only female mice to avoid complications due to aggression, which is often observed amongst male mice. These strains were selected as they reflect the most common inbred (C57/Bl/6) and outbred (ICR(CD-1)) mouse strains used in UK laboratories (Westwood, 2007). The mice were obtained from Harlan UK (Bicester, UK) aged six weeks old and were supplied over three batches (batches 1 and 2, $n = 48$; batch 3, $n = 32$). The mice had been reared in conventional cages containing sawdust and shredded paper for nesting material. Upon arrival, the mice were randomly assigned to groups of four and housed in conventional, opaque polypropylene laboratory cages (dimensions 37 cm × 21 cm × 15 cm, $l \times w \times h$) with sawdust substrate (Lignocel, IPS, UK) and approximately 10 cm^3-shredded paper nesting material, which was changed weekly. All the cages were supplied with standard expanded rat and mouse diet (IPS, UK) and tap water *ad libitum*, and the mice were housed on a reverse 12:12 light–dark cycle (lights off 13.00 h). The room was maintained at a temperature between 19 and 23 °C and during the dark phase it was lit by a 25 W red light.

The cages were positioned on tables (0.71 m above the ground) to allow easy installation of video cameras suspended above the cages and to minimise disturbances to the cages during daily checks. Cages were distributed systematically to avoid any position bias.

From the age of eight weeks, each cage of mice was given a different potential enrichment for one week (see Appendix A), over a period of 12 weeks. The PEs were presented in a pseudo-randomised order of presentation (the same PE was not presented twice to a cage of mice), such that each cage received 12 different PEs from a pool of 24, selected by a panel of experts as likely to have the greatest biological relevance to the mice. Due to the total number of PEs available (24), these were presented in a non-balanced design. The mice were individually marked using hair-bleach or hair dye.

2.2. Behavioural observations

Observations were taken by one observer (SB) and were categorised as shown in Table 1. A distinction was drawn between behaviours that involved the PE and those that did not. If PEs were shredded and incorporated substantially into the nest, then the modified nest was itself categorised as a PE. Behaviour was recorded on three occasions during the week (Day 1 (PE put into cage), Day 3 and Day 6). Instantaneous scans of all the individuals in the study were taken on the last hour of the light phase and on the second, third and fourth hour of the dark phase from video (one scan per hour). All results were converted from scans to

Table 1
Description of the behaviours recorded in the study, and % of total time-budget for both strains of mice for the 12 weeks of the study

Behaviour	Description	% Time
Aggression	Biting, chasing, pinning or mounting. Actor or recipient	0.07
Bar chew	Chewing bars of cage	0.25
Rest in cage	Sitting or lying for >10 min in same position in cage or nest. May include small body movements such as turning	0.25
Rest in PE	As above, but in or on PE	0.25
Climb PE	Locomotor activity on sides of PE, including climbing, hanging or brief stationary periods	0.5
Dig	Pushing material forwards or backwards with nose, forepaws or hind legs	0.5
Manipulate	Manipulating any cage materials, except PE, with mouth or paws.	0.5
Rear PE	Rearing with hind legs in contact with PE	0.5
Sniff PE	Sniffing whilst in contact with the PE.	0.5
Social interaction	Sniffing or allo-grooming conspecific. Actor or recipient	0.5
Feed from substrate	Feeding from food placed in substrate or removed from hopper or PE	0.9
Drink	Licking water bottle	1.0
Locomotion in PE	Locomotion on or inside PE (excluding climbing)	1.25
Stationary in PE	Body stationary on or inside PE, head or tail may move	1.25
Manipulate PE	Manipulating PE, with mouth or paws.	1.75
Rear in cage	Rearing with hind feet in cage	2.0
Sniff cage	Sniffing whilst not in contact with the PE	2.02
Groom self	Shaking, licking, wiping or scratching any part of own body	2.1
Locomotion in cage	Locomotion in cage (excluding climbing and locomotion in PE)	2.75
Stationary in cage	Body stationary in cage, excluding PE, head or tail may move	2.75
Locomotion stereotypic	Repeated invariant patterns of locomotion	5.5
Feed from hopper	Gnawing or manipulating pellets in food hopper with paws or mouth	7.8
Climb bar	Non-stereotypic locomotion on cage lid	8.5
Hidden in PE	Head hidden from observer's view when inside or in contact with PE	15.0
Hidden in cage	Head hidden from observer's view when not inside or in contact with PE, usually in nest or nest under hopper	41.25

average individual percentages of time allocated to each activity by dividing by 4 (number of mice in each cage) and multiplying by 100.

2.3. Statistical analysis

We performed a preliminary screen of all 24 PEs separately and subsequent cluster analysis allowed us to make more general conclusions. An overall average value across the four scans was taken for each cage on each of the three observation days, and used in subsequent analysis. The 24 separate PEs were categorised post-hoc according to five independent physical features. These features were: overall volume assessed using a water displacement test (continuous), whether they had an internal component that mice could potentially enter (categorical: two levels; enter or not enter), physical presence and texture (categorical: three levels; outside cage, inside cage and hard, inside cage and soft), food value (categorical: three levels; no energy value, minimal energy value, energy value approximating to normal feed), and frequency of replacement or repair by staff during the week (categorical: three levels; not replaced or repaired during week, replaced or repaired once during week, replaced or repaired more than once during week). A number of additional physical classification features were examined, but these were statistically redundant and subsumed by the five primary features.

A cluster analysis was conducted on the 24 PEs according to these five features, using the two-set cluster procedure in SPSS, which creates clusters based on both continuous and categorical variables. The procedure began with the construction of a cluster features (CF) Tree by placing the first PE (case) at the root of the tree in a leaf node that contains variable information about that case. Each successive case was then added to an existing node or formed a new node, based upon its similarity to existing nodes and using the distance measure as the similarity criterion. A node that contained multiple cases contained a summary of variable information about those cases. Thus, the CF tree provided a capsule summary of the data file. The leaf nodes of the CF tree were then grouped using an agglomerative clustering algorithm. To determine which number of clusters was ''best'' (i.e., minimised distances between clusters), each cluster solution was compared using Schwarz's Bayesian criterion (BIC) as the clustering criterion. In this case, the smallest BIC criterion (169.1) was obtained with three clusters. There was also a relatively large ratio of distance measures (1.92) on moving from a 2-cluster to a 3-cluster solution. The 3-cluster solution assigned 4 PEs to the first cluster, 9 to the second and 11 to the third. Examination of the mean and standard deviation of the features for each cluster showed that Cluster 1 (edible/large/replaced) comprised PEs that were edible, occupied more than average internal space within the cage, needed repair or replacement during the week, and sometimes provided internal space. Cluster 2 (shelter/hard/outside) comprised PEs that sometimes provided internal space, were hard or outside the cage, were not replaced or repaired during the week, and provided little or no food value. Cluster 3 (soft/replaced/chewable) comprised PEs that provided no internal space, had soft material components, usually needed to be replaced or repaired during the week, and were generally chewable but with little food value. Details of the PE of each cluster are given in Appendix A.

A generalised linear model was employed using SPSS to examine the between-subjects effects of strain and the interaction between strain and PE cluster on behaviour. Average values across the individual PE within a cluster were taken for each cage to avoid false replication. Where there were no strain $\times$ PE cluster interactions, the between-subjects effects of PE cluster were examined. Post-hoc analysis of differences between means for the three PE clusters was conducted using Tukey's honestly significant difference tests. Where strongly significant effects of PE cluster were detected on particular behaviours (at $p < 0.01$), the contribution of each individual PE was examined using repeated measures GLM on each cluster separately, followed by post-hoc analysis using Tukey's honestly significant difference tests.

Day of the week was included in the model as a repeated measures within-subjects effect using a univariate, mixed model. The assumption that the variance-covariance matrices were the same across the cells formed by the between-subjects effects was tested using Mauchly's test of sphericity. If the assumption was not met then, the degrees of freedom were adjusted to validate the F-statistic used in determining the significance of within-subjects effects. The interactions between PE, strain and day were then examined.

3. Results

The average time allocated to each activity is shown in Table 1. The majority of time was spent hidden in the nest or the PE. Substantial amounts of time were spent feeding, bar climbing and in stereotypic locomotion. All other activities occupied less than 3% of time.

A number of behaviours were influenced by strain (Table 2). The outbred mice performed more bar chewing, digging, manipulation of PEs, and rearing in the cage. They were also more likely to be hidden in the cage. The inbred mice performed more drinking, cage sniffing and social interaction, and more than three times as much bar climbing as the outbred mice.

The most frequently reared on PEs were the grass (1.83% of time), hemp fibre mats (1.68%), baking potato (1.4%) and the hammock (1.18%). The most frequently rested on PEs were the hammock (2.6%), mealworms stimulus (1.05%), Z partition (1.05%), and the teabag (0.9%). There were no significant effects due to strain or time (habituation) for these behaviours in relation to these PEs.

Many of the strain effects on behaviour depended on the environment, as indicated by PE cluster × strain interactions (Fig. 1). Outbred mice were differentially more likely to manipulate PEs in the soft/replaced/chewable cluster ($F(2, 87) = 4.5$; $p < 0.05$), and to be hidden within them ($F(2, 87) = 9.16$; $p < 0.001$). In the presence of PEs in Clusters 1 (edible/large/replaced) and 2 (shelter/hard/outside), the outbred mice spent more time hidden in the cage than the inbred mice ($F(2, 87) = 4.25$; $p < 0.05$). Both strains reduced the time spent hidden in the cage in the presence of PEs in the soft/replaced/chewable cluster to a similar level (see Fig. 1). The inbred mice performed differentially more social interaction in the presence of the food-related PEs in the edible/large/replaced cluster ($F(2, 87) = 3.6$; $p < 0.05$).

Some between-subjects effects of PE cluster did not depend on strain (Table 3). Mice performed more feeding from the substrate in response to the edible/large/replaced cluster PEs than the other clusters. When the PEs within the edible/large/replaced cluster were examined separately for their effects on these behaviours, the hamster food resulted in more substrate feeding than the food block or the potato ($F(3, 46) = 6.80$; $p = 0.001$). The mice performed more locomotion on the PEs in the shelter/hard/outside cluster than the other clusters, primarily using the running disc of the igloo shelter ($F(8, 117) = 84.4$; $p < 0.001$). Potential enrichments in the soft/replaced/chewable cluster provoked the greatest variety of behaviour. Mice were more often stationary within the PEs in the soft/replaced/chewable cluster and used these for sniffing, climbing, and resting more than the PEs in the other clusters (Table 3). When the PEs within the soft/replaced/chewable cluster were examined separately, climbing differed between the PEs

Table 2
Significant effects of Strain on % time allocation to different behaviours

Behaviour	Mean C57/Bl/6	Mean ICR(CD-1)	Pooled S.E.M.	F-value d.f. 1, 87	p
Bar chew	0.03	0.68	0.08	32.0	<0.001
Bar climb	13.08	3.75	0.48	107.4	<0.001
Dig	0.38	0.73	0.10	7.7	<0.01
Drink	1.33	0.68	0.10	17.4	<0.001
Manipulate PE	1.00	2.38	0.23	16.8	<0.001
Hidden in the cage	38.70	44.08	1.48	16.4	<0.001
Rear cage	1.60	2.15	0.15	6.8	=0.01
Sniff cage	2.23	1.05	0.18	17.4	<0.001
Social interaction	0.75	0.45	0.10	4.9	<0.05

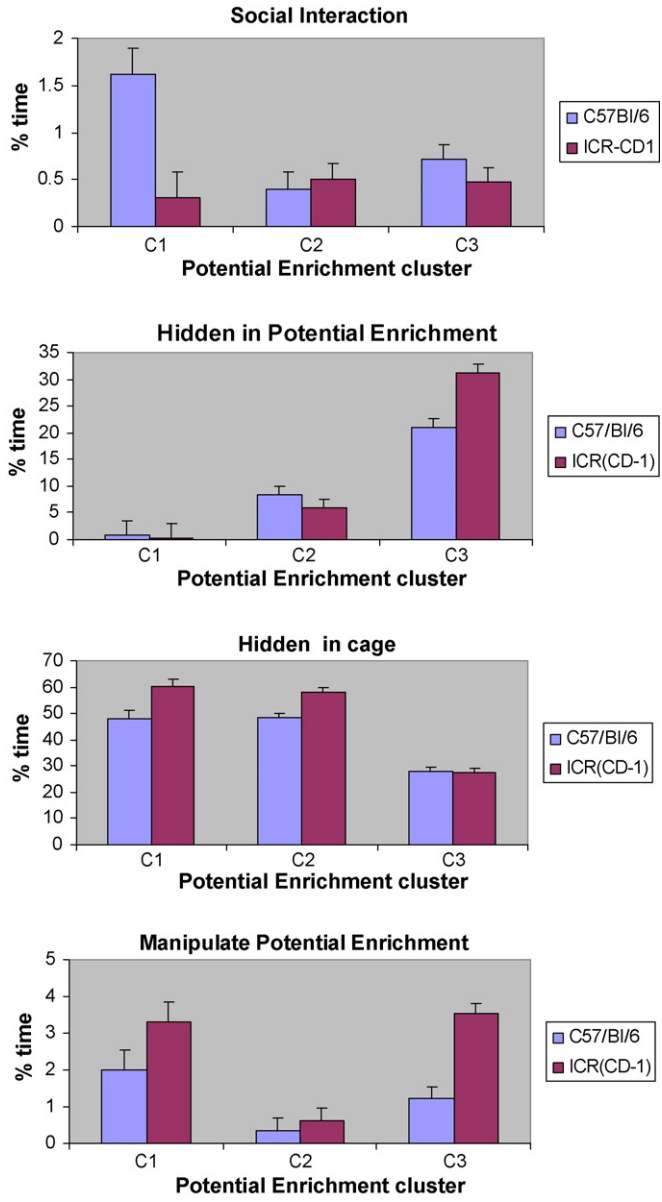

Fig. 1. Significant interactions between strain and potential enrichment cluster. Bars are S.E.M. (Cluster 1 = edible/large/replaced; Cluster 2 = shelter/hard/outside; Cluster 3 = soft/replaced/chewable).

($F(10, 144) = 10.04$; $p < 0.001$) with most climbing on the Hessian curtain. Sniffing differed between PEs ($F(10, 144) = 7.61$; $p < 0.001$) with the grass and hemp mat stimulating more sniffing than most of the other PEs. Being stationary in the PEs differed between PEs ($F(10, 144) = 10.6$; $p < 0.001$) with the mice being stationary in the hammocks more than in any other PE.

Table 3
Significant effects of potential enrichment cluster, with no strain interaction, on % time allocated to different behaviours

Behaviour	Cluster 1 edible/ large/replaced	Cluster 2 shelter/ hard/outside	Cluster 3 soft/ replaced/chewable	*F*-ratio d.f. 2, 87	*p*
Climb PE	0.13 ± 0.10 a	0.18 ± 0.10 a	0.88 ± 0.10 b	18.94	<0.001
Feed from substrate	2.15 ± 0.20 a	0.80 ± 0.20 b	0.60 ± 0.20 b	17.10	<0.001
Locomotion in PE	0.35 ± 0.25 a	1.80 ± 0.22 b	0.82 ± 0.22 a	10.54	<0.001
Rest in PE	0.23 ± 0.23 a	0.00 ± 0.15 a	0.68 ± 0.13 b	13.79	<0.001
Sniff PE	0.15 ± 0.18 a	0.15 ± 0.13 a	0.95 ± 0.10 b	25.63	<0.001
Stationary in PE	0.55 ± 0.30 a	0.68 ± 0.20 a	1.85 ± 0.18 b	13.32	<0.001

Values are means ± S.E.M. Values in rows with different letters (a, b), differ significantly from each other at $p < 0.05$

Table 4
Significant effects of Day, with no strain interaction, on % time allocated to different behaviours

Behaviour	Day 1	Day 3	Day 6	*F*	Adjusted d.f.	*p*
Stereotypic locomotion	4.20 ± 0.34	6.18 ± 0.45	6.20 ± 0.43	6.4	1.98, 172	<0.01
Manipulate other	0.95 ± 0.15	0.40 ± 0.08	0.28 ± 0.08	10.4	1.49, 129	<0.001
Hidden in PE	4.1 ± 0.53	18.83 ± 1.40	22.23 ± 1.48	92.7	1.81, 157	<0.001
Rear cage	2.55 ± 0.23	1.55 ± 0.15	1.53 ± 0.15	10.8	1.82, 157	<0.001
Sniff cage	2.43 ± 0.25	1.55 ± 0.20	0.90 ± 0.13	15.2	1.76, 153	<0.001
Sniff PE	1.05 ± 0.05	0.35 ± 0.10	0.15 ± 0.05	14.6	1.68, 146	<0.001
Stationary in cage	3.78 ± 0.30	2.33 ± 0.23	2.13 ± 0.25	16.9	1.81, 158	<0.001
Stationary in PE	1.65 ± 0.20	1.15 ± 0.20	0.83 ± 0.15	9.1	1.88, 164	<0.001

Values are means ± S.E.M.

For an enrichment to be successful, animals should not habituate to it. We, therefore, considered the effects of time on the behaviour of the mice. The strains differed in the time-course of their behaviour across each weekly environmental change. The within-subjects analysis of day revealed many changes in behaviour across each week. These are presented in Table 4; all other differences were non-significant. Linear decreases were observed in manipulation, rearing in the cage, sniffing the cage, sniffing the PE, being stationary in the cage and being stationary in the PE. Linear increases in stereotypic locomotion and time spent hidden within the PE occurred over the course of each week. Strain × day interactions related to cage locomotion ($F(2, 348) = 4.2$; $p = 0.016$) and manipulating the PE ($F(2, 348) = 5.86$; $p = 0.003$) where the decline across days was more pronounced for outbred than inbred mice (Fig. 2). In addition, a strain × day interaction occurred ($F(2, 348) = 7.63$; $p = 0.001$) for being hidden in the cage where a decline across days was noted for inbred mice, but a slight increase occurred in outbred mice. PE cluster × day interactions related to feeding from the substrate, where the biggest decline from day 1 was noted for the edible/large/replaced cluster PEs ($F(3.8, 166) = 3.9$; $p < 0.01$), and sniffing at the PEs, which declined from Day 1 for the soft/replaced/chewable cluster PEs but was highest on Day 2 for the edible/large/replaced cluster PEs ($F(3.4, 146) = 10.3$; $p < 0.001$).

4. Discussion

The primary objective of this study was to investigate the behavioural responses of mice to a range of potential enrichments so that we could elucidate which characteristics were most

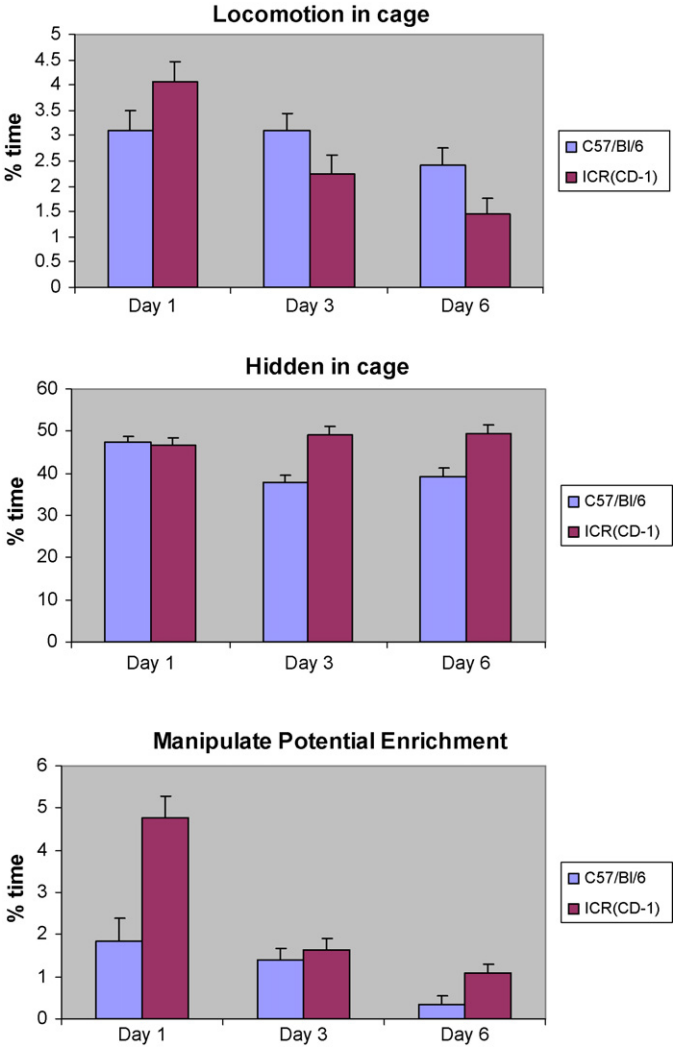

Fig. 2. Significant interactions between strain and observation day. Bars are S.E.M.

beneficial (biologically relevant) and incorporate these into a single, universal enrichment for laboratory mice. A universally acceptable enrichment will increase the occurrence of non-deleterious behaviours, that is, broaden the repertoire of normal behaviour, have a significant effect for both strains, and the mice will not habituate to it. Two behaviours indicated that PEs might have had all these three characteristics. Rearing on the PE and resting on the PE were both significantly increased for some of the enrichments, and there was no effect of strain or habituation. The most frequently reared on PEs were the grass, hemp fibre, baking potato and the hammock. The most frequently rested on PEs were the hammock, mealworms stimulus, Z partition, and the teabag. The hammock is the only PE to appear in both these lists, indicating it encouraged rearing and resting behaviour the most of all the PEs without being subject to strain differences or habituation. There are no clear negative consequences of increased rearing or

resting, and one could argue that increased rearing may represent some 'positive' enhancement of 'exploratory' activity or increased behavioural repertoire. The cluster analysis shows that Cluster 1 PEs (primarily PEs that were edible) had the greatest influence on manipulating the PEs for both strains (Fig. 1) and the largest effect on feeding from the substrate (Table 3) confirming earlier work (e.g., Chamove, 1989). Therefore, we suggest that a universal enrichment for laboratory mice would combine those features that increased the presumed positive behaviours without having strain or habituation effects (i.e., a Hessian hammock) with those features that the cluster analysis revealed had the greatest effects on presumed positive behaviours (i.e., incorporated food). This enrichment has been developed and behavioural responses to it will be reported separately. Overall, the times spent on basic maintenance behaviours such as feeding from the hopper, drinking, self-grooming, social behaviour, resting in the cage and locomotion in cage were not significantly affected by PE type. The fact that such basic functions were not perturbed by small changes in cage environment might allay some concerns about the effects of enrichment on standardisation for experimental purposes (e.g., Tsai et al., 2002). However, it also raises concerns about the value of these PEs in improving welfare. None of the PEs produced a significant reduction in stereotypic or bar-related activities, which were relatively high under all conditions, particularly in the C57/Bl/6 mice. Similarly, none of the PEs reduced aggression, although this might have been due to the very low levels often seen in groups of females. The effects of the PEs on the behaviour of male mice was not examined here, so generalisation of these findings should be made cautiously, but a reduction in aggression amongst males is a possibility (but see McGregor and Ayling, 1990; Haemisch et al., 1994).

Despite these reservations, PE type influenced the performance or location of a number of behaviours in ways that might have a positive welfare consequence, although additional studies using a range of welfare indicators would be needed to draw firm conclusions on this (Mendl, 2001). The PEs in the soft/replaced/chewable cluster promoted the most diverse overall time-budget, with increased climbing, sniffing and resting directed at these PEs, as well as increased time being stationary or hidden within them, compared with edible/large/replaced cluster and shelter/hard/outside PEs. The time spent hidden in the conventional nest reduced correspondingly in the presence of soft/replaced/chewable cluster PEs, suggesting the mice had a higher relative preference for some features of the PEs. Strain × PE interactions showed that the ICR(CD-1) mice were more sensitive to features of potential hiding places than the inbred mice. Potential enrichments in Clusters 1 (edible/large/replaced) and 2 (shelter/hard/outside) provided few hiding places and, when these PEs were present, the ICR(CD-1) mice spent more time than the active C57/Bl/6 mice in the conventional nests. However, in the presence of the soft/replaced/chewable cluster PEs, the ICR(CD-1) mice shifted their hiding location dramatically from the conventional nests to the PEs (Fig. 1). The ICR(CD-1) mice also manipulated the edible/large/replaced cluster and soft/replaced/chewable cluster PEs more than the C57/Bl/6 mice, often shredding them and incorporating them into modified nests. The PEs in the edible/large/replaced cluster stimulated increased substrate feeding in both strains, almost certainly due to the olfactory and gustatory cues they provided.

Previous studies have drawn a distinction between state and trait anxiety. State anxiety is demonstrated in tests such as the open field, elevated plus maze or light/dark choice test, where the animal is placed involuntarily in a novel situation, whereas underlying trait anxiety is revealed by observation of free or spontaneous exploration (Kopp et al., 1999). Studies comparing performance on these tests have characterised the C57 mice as showing relatively low state anxiety (especially if differences in baseline locomotor activity are factored out) but high trait anxiety in comparison with CBA and BALB/c strains (Avgustinovich et al., 2000). Chapillon

et al. (1999) found that early environmental enrichment further reduced state anxiety but not trait anxiety in C57 mice.

In contrast to this background information, there have been few comparative behavioural studies of outbred mice. Adams et al. (2002) compared the characteristics of the CD-1 strain and its ICR substrain. They found that the ICR mice had a severe visual impediment that reduced performance in tests where learning of visual cues was required. In comparison with inbred strains, outbred Swiss CD-1 mice exhibit low levels of anxiety (Parmigiani et al., 1999). A number of studies have shown that bar-related and bar-chewing behaviour are exhibited at a high level in the ICR(CD-1) strain, and that this behaviour represents an attempt to escape from the cage environment (Nevison et al., 1999a; Lewis and Hurst, 2004).

Based on this previous work, we expected and found higher locomotor activity in the C57/Bl/6 mice, manifested primarily as bar-climbing activity. The C57/Bl/6 mice spent over 13% of their time performing bar-climbing compared with only 4% by the ICR(CD-1) mice. Bar-climbing in ICR(CD-1) mice has been shown experimentally to represent an attempt to escape from the cage environment (Nevison et al., 1999a; Lewis and Hurst, 2004). The increased locomotor activity of the C57/Bl/6 mice was primarily at the expense of time spent hidden in the cage. Direct observations suggested that mice were generally resting when they were hidden in the cage, although it was impossible to quantify this precisely. An unexpected strain difference was the increased time spent in sniffing and social interaction by the C57/Bl/6 mice. This has not previously been reported.

Each PE was provided for a period of just one week. On the first day of presentation, mice responded with an initial exploration of both the PEs and the cage, indicated by increased rearing, sniffing cage, sniffing the PE, manipulation of the PE and manipulation of other parts of cage. On the first day of presentation of a new PE, the time spent in being hidden or performing stereotypic locomotion was lower than on subsequent days. By the third day of exposure, exploration had generally reduced and there was a pronounced increase in the time spent hidden in the PEs, from 4% to 19%, which then stabilised.

Examination of strain responses over time indicated that the increased manipulation of PEs shown by the ICR(CD-1) mice (primarily to the soft/replaced/chewable cluster PEs) was an initial exploratory response that was most apparent on Day 1. This suggests that the ICR(CD-1) mice were less anxious and neo-phobic than the C57/Bl/6 mice, as initially predicted. If C57/Bl/6 have high trait anxiety, as suggested by previous studies (Avgustinovich et al., 2000), then they would not be expected to leave a familiar area of the cage to explore a novel area or object. The design of this experiment, with a weekly change in PE type, might have benefited the low trait anxiety ICR(CD-1) mice more than the C57/Bl/6 mice. Indeed the high levels of bar-climbing may even have been an expression of attempting to escape from continual novelty. We suggest that the C57/Bl/6 mice might respond better to a more stable enriched environment.

In conclusion, the examination of the behavioural responses of mice to a range of potential enrichments allowed us to determine which characteristics of these were the most biologically relevant to the mice, and which might be incorporated into a universal enrichment. Our study also emphasizes that in any such enrichment of the cage environment, strain differences might need to be taken into account. Attempts should be made to devise universal enrichments that improve welfare across a wide range of strains and for both sexes, however, if this is not possible then enrichment might need to be implemented in a strain-specific manner. Low anxiety, exploratory strains might benefit from the provision of novel objects to manipulate or to use as preferred hiding places, but strains exhibiting high trait anxiety might require a more stable cage environment.

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Appendix A. Description of the potential enrichments

Note: Several of the potential enrichments were 'fixed' in their position on the cage floor by attaching them to the cage lid. This was to prevent the PE being moved and causing flooding, but the fixing method usually allowed a small amount of movement of the PE.

A.1. CLUSTER 1 (edible/large/replaced)

Hamster food: A food for pet hamsters containing a range of nuts and seeds (''Harry Hamster'' Premium Complete Food, Supreme Pet Foods Limited, UK) sprinkled in the substrate. Approximately 39 g was scattered on the floor and raked into the substrate with fingers.

Food block: A 3.5 cm × 3.5 cm × 7.0 cm Aspen wood block (Lillico Biotechnology, UK) with eight, 0.2–0.5 cm holes drilled in each side and filled with peanut butter (Sunpat Smooth Peanut Butter, Premier Ambient Foods Ltd, UK) placed in the centre of the cage.

Potato: A washed and dried baking potato (either Maris Piper or Marfona), approximately 275 g and 10 cm long, fixed in a central position on the cage floor by attaching it to the cage lid with a three cable ties.

Seed brush: A 10 cm, wooden scrubbing brush with natural bristles between which was inserted approximately 13 g of Hamster food (described above). The seed brush was placed in the centre of the cage.

A.2. CLUSTER 2 (shelter/hard/outside)

Control: No potential enrichments added to the cage. A gloved hand was placed in the cage for approximately 30 s on replenishment days to simulate the replenishment of a potential enrichment.

Coconut shell: A non-husked, green 'jelly' coconut was sawed in half, pithed, dried at 20°C for at least 24 h and a 3 cm 'entrance' hole cut into the shell. The coconut shell was placed centrally in the cage with the sawed surface downwards, fixed in position by attaching it to the cage lid with a screw-ring and one large paper clip.

Shadows: A 15 cm diameter white cardboard disc with leaf shaped holes covered in yellow, blue, pink and white tissue paper, rotating at 1 revolution/minute approximately 22 cm above the cage. The moving shadows produced by the disc covered approximately 80 cm^2 at the rear of the cage.

Mirror: A highly polished, steel plate mirror (8 cm × 7 cm) adapted from an enrichment for pet birds (Product code LAM 4242, Ferplast Pet Products, UK), suspended from the cage lid by a length of chain such that the bottom of the mirror rested on the floor and the back against the rear wall of the cage.

Birdsong: A CD of various songbird songs downloaded from the internet, played back through a personal CD player and two miniature earphones mounted on the rear corners of the cage. The volume was adjusted to be only just audible to humans at the front of the cage. The birdsongs were played-back on a random basis from 11 a.m.to 3 p.m., five days each week.

Maze cube: A cube (15 cm × 15 cm × 15 cm) made from translucent white, corrugated plastic (Corex lids, Key Industrial Equipment Ltd, UK), with several openings and levels within

it. The maze cube was fixed in a central position on the cage floor by attaching it to the cage lid with a single cable tie.

Half log: The outside (bark) portion of half a log approximately 10 cm long and 5 cm in radius (Small, Habba Hut, Zoo Med Europe, Belgium). The half log was fixed in a central position on the cage floor by attaching it to the cage lid with a screw-ring and two large paper clips.

Igloo shelter: An amber, polycarbonate Igloo-shaped shelter (10 cm diameter, 6 cm high) with a 15 cm diameter running disc mounted on top of the shelter (Fast-trac; Bioserv, USA). The shelter was placed in the centre of the cage.

Rawhide chew: A rawhide leather chew (10 cm × 1.5 cm, approximately 23 g) designed as a chewing enrichment for dogs (Goodboy dog chews, Den-marketing, UK), placed in the centre of the cage.

A.3. CLUSTER 3 (soft/replaced/chewable)

Mealworms stimulus: A 5 cm^2 piece of Hessian cloth was placed in a tub of fresh mealworms for 24 h and then suspended against the rear wall of the cage by one paper clip attached to the cage lid.

Teabag: An empty, herbal flavoured teabag (cranberry, raspberry and elderflower flavour, Twinings, UK) suspended against the rear wall of the cage by one paper clip attached to the cage lid.

Climbing rope: A cotton rope, 12 cm long and 1.8 cm thick with an 8 cm long wooden perch 9 cm from the top (''Pet love percher'', Pet Love, UK) modified from an enrichment for pet parrots. This was suspended centrally in the cage by one paperclip.

Hammock: A 17 cm × 19 cm piece of Hessian cloth (The Fabric Shop Ltd, UK) suspended at each corner by paperclips from the lid across the width of the cage at the rear wall. The paperclips across the width of the hammock were 8 cm apart.

Grass: A 20 cm × 10 cm sod of grass (Lawn turf, local garden centre) placed at the rear of the cage.

Coir flowerpot: A flowerpot (8.5 cm diameter at opening, 5.5 cm at the base and 8 cm high) made of coir, a material manufactured from coconut fibres (Unwins Peat Free Coir Pots, Unwins Seeds Ltd, UK).

Curtain: A 14 cm × 18 cm piece of Hessian cloth (The Fabric Shop Ltd, UK) suspended from the lid bars along the centre-line of the length of cage by two paperclips such that it formed a curtain reaching the floor of the cage.

Z partition: An opaque, black Perspex rectangle bent to form a 'Z' shape. The central section was 15 cm long and the end sections 5 cm long; all were 10 cm high. There were 2.5 cm × 2.0 cm 'castellations' along the top edge of the partition. The central section had a doorway (5 cm × 2.5 cm) and seven 0.5 cm diameter holes drilled into the partition. One end section was covered with Hessian material and the other section had a doorway (3.0 cm × 2.5 cm). The Z partition was placed diagonally in the cage and was fixed in place on the floor by being clipped to the lid.

Hemp mat: A 10 cm × 20 cm × 1 cm hemp fibre mat (Hemcore Ltd, UK), placed on the floor at the rear of the cage.

Mice stimulus: A 5 cm^2 piece of Hessian material (The fabric shop Ltd, UK) was placed in a bag of substrate removed from the latrine area of a cage containing non-familiar mice of the same sex and strain of the test mice for 24 h. When placed in the test cage, the Hessian square was suspended against the rear wall of the cage by a paper clip attached to the bars of the lid.

Digging pot: A ceramic bowl (8 cm diameter, 3.5 cm deep, Mason Cash, UK) filled and compacted with organic compost (Gem natural earth range, organic peat free compost, Joseph Metcalf, UK.)

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